Expanded View Figures

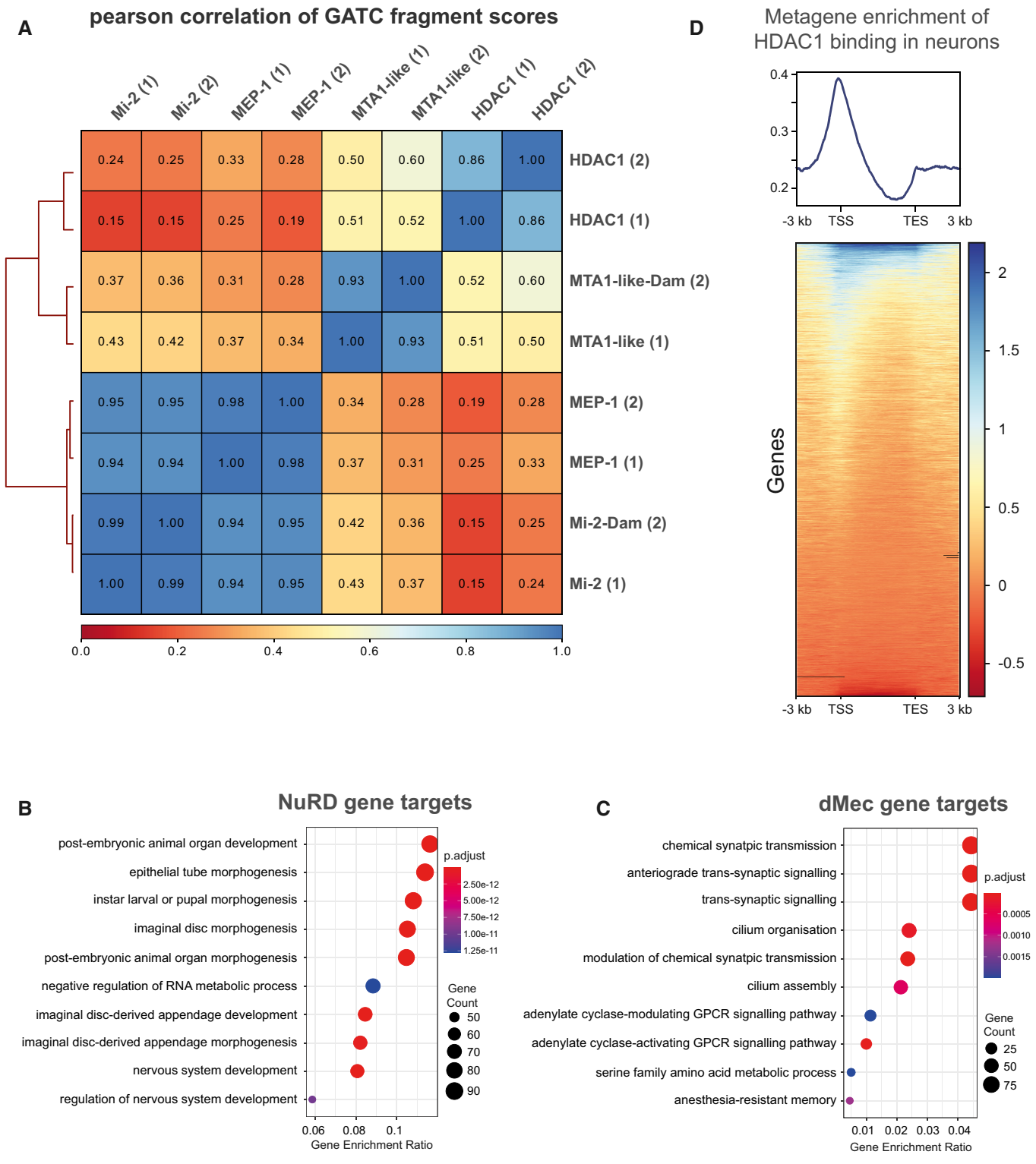

Figure EV1.

Figure EV1. Genomic binding of NuRD components.

A Correlation between NuRD subunits (including replicates) in larval neurons (*elav*-GAL4). Very strong correlations are seen between Mi-2 and MEP-1 binding while the correlation between Mi-2/MEP-1 and either MTA-1like or HDAC1 is relatively lower. Good correlations are exhibited between replicates for each subunit (spearman's correlation $r^2 = 0.85–0.95$).

B GO analysis of NuRD gene targets.

C GO analysis of dMec gene targets genes.

D Metagene analysis of HDAC1 binding. TSS is Transcriptional Start Site, and TES is Transcriptional End Site.

Figure EV2. RNA-seq quality control and differentially expressed genes.

A Heatmap showing Pearson correlation of RNA-seq reads between biological replicates and control and experimental groups. Very strong correlations are observed between replicates ($R^2 > 0.97$) with slightly lower correlations between knockdown and controls reflecting the changes in *Mi-2* RNAi transcriptome.

B Genome browser tracks indicating RNA-seq read coverage at the *Mi-2* locus. *Mi-2* reads are clearly depleted in *Mi-2 RNAi* compared with *elav*-GAL4 × *mCherry RNAi* controls (adjusted *P* value $< 2.2 \times 10^{-227}$).

C Heatmap showing relative changes in normalised gene expression for the thirty most significantly upregulated genes.

D Heatmap showing relative changes in normalised gene expression for the 30 most significantly down-regulated genes.

E, F Read coverage for (E) *gammaTub37C* and (F) *RpS5b* loci, which are non-CNS genes ectopically expressed in *Mi-2* knockdown.

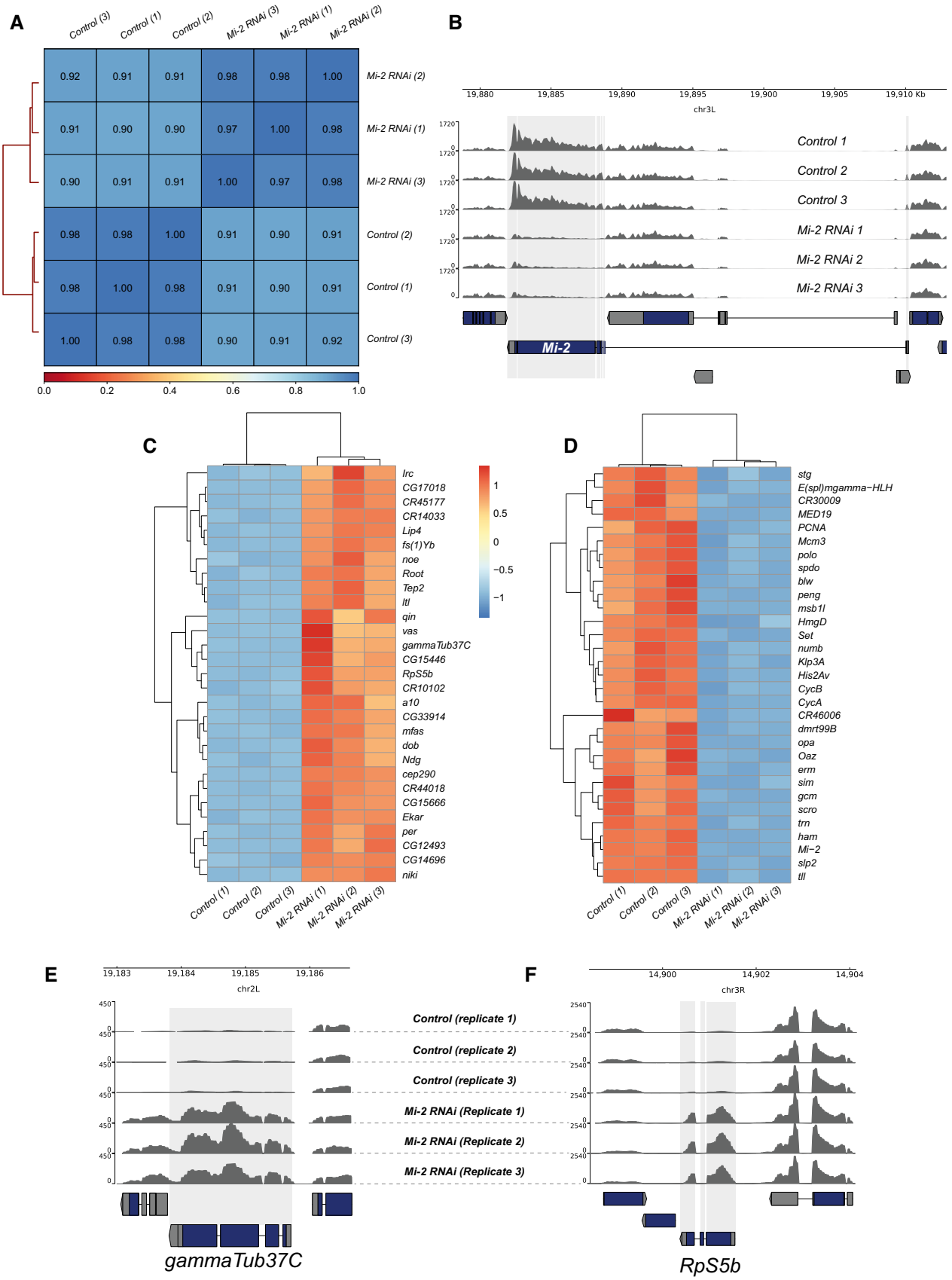
A
Control (3)
Control (1)
Control (2)
Mi-2 RNAi (3)
Mi-2 RNAi (1)
Mi-2 RNAi (2)
0.92 0.91 0.91 0.98 0.98 1.00 Mi-2 RNAi (2)
0.91 0.90 0.90 0.97 1.00 0.98 Mi-2 RNAi (1)
0.90 0.91 0.91 1.00 0.97 0.98 Mi-2 RNAi (3)
0.98 0.98 1.00 0.91 0.90 0.91 Control (2)
0.98 1.00 0.98 0.91 0.90 0.91 Control (1)
1.00 0.98 0.98 0.90 0.91 0.92 Control (3)
0.0 0.2 0.4 0.6 0.8 1.0
B
19,880 19,885 19,890 19,895 19,900 19,905 19,910 Kb
chr3L
Control 1
Control 2
Control 3
Mi-2 RNAi 1
Mi-2 RNAi 2
Mi-2 RNAi 3
Mi-2
C
lrc
CG17018
CR45177
CR14033
Lip4
fs(1)Yb
noe
Root
Tep2
ltl
qin
vas
gammaTub37C
CG15446
RpS5b
CR10102
a10
CG33914
mfas
dob
Ndg
cep290
CR44018
CG15666
Ekar
per
CG12493
CG14696
niki
1
0.5
0
−0.5
−1
Control (1) Control (2) Control (3) Mi-2 RNAi (1) Mi-2 RNAi (2) Mi-2 RNAi (3)
D
stg
E(spl)mgamma−HLH
CR30009
MED19
PCNA
Mcm3
polo
spdo
blw
peng
msb1l
HmgD
Set
numb
Klp3A
His2Av
CycB
CycA
CR46006
dmrt99B
opa
Oaz
erm
sim
gcm
scro
trn
ham
Mi−2
slp2
tll
Control (1) Control (2) Control (3) Mi-2 RNAi (1) Mi-2 RNAi (2) Mi-2 RNAi (3)
E
19,183 19,184 19,185 19,186
chr2L
Control (replicate 1)
Control (replicate 2)
Control (replicate 3)
Mi-2 RNAi (Replicate 1)
Mi-2 RNAi (Replicate 2)
Mi-2 RNAi (Replicate 3)
gammaTub37C
F
14,900 14,902 14,904
chr3R
RpS5b

Figure EV2.

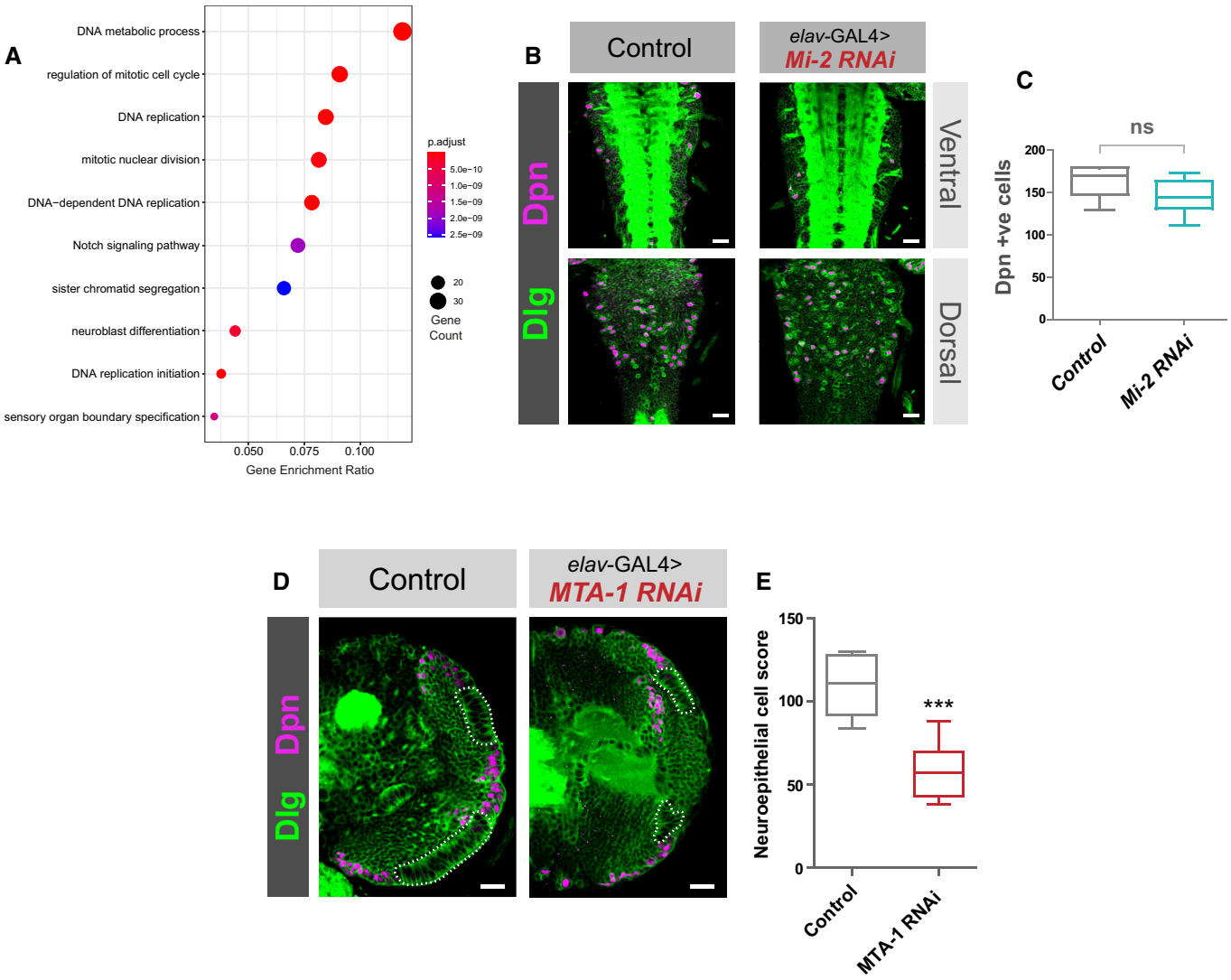

Figure EV3. *NuRD* knockdown larval brain phenotypes.

A GO analysis of downregulated genes.

B *Mi-2* knockdown does not affect NSC numbers in the VNC. (B) Example images from ventral and dorsal sections of the VNC. Scale bars = 20 μm.

C Quantification of Dpn-positive cells (no significant difference). Six VNCs measured for each genotype. No significant difference (two-tailed student's *t*-test). Represented as a box plot.

D *MTA1-like* knockdown causes an optic lobe phenotype. (D) Representative images of control (*elav*-GAL4;; *mCherry RNAi*), and *MTA1-like* knockdown in the third instar larval CNS (all scale bars = 20 μm). Discs large (Dlg) = green, Deadpan (Dpn) = magenta. Neuroepithelial cells are highlighted by dashed lines.

E Quantification of optic lobe neuroepithelial cells of genotypes shown in panel A. At least eight brains measured for each genotype. ***$P < 0.001$ (one-tailed student's *t*-test). Represented as a box plot.

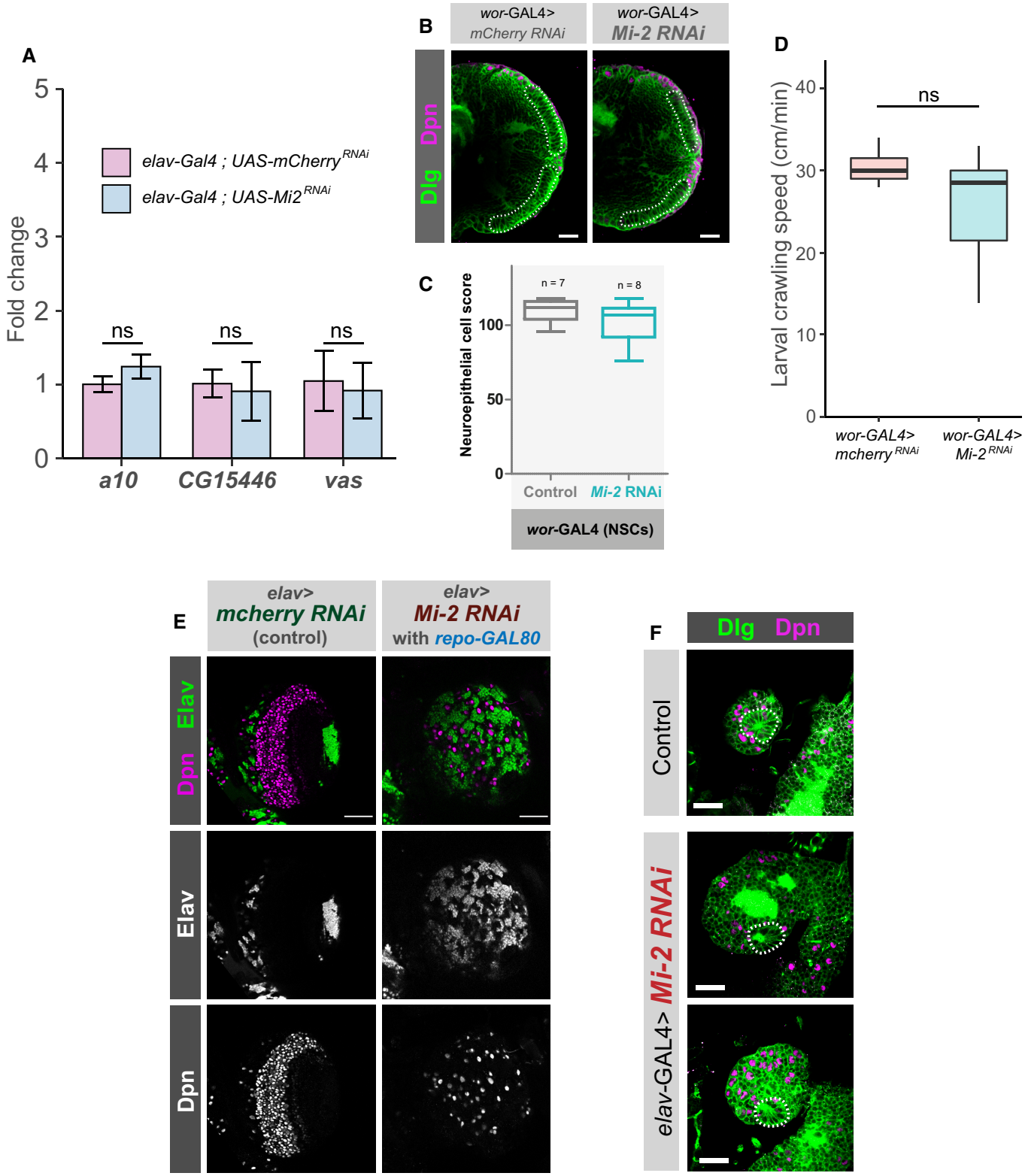
A
Fold change
elav-Gal4 ; UAS-mCherry RNAi
elav-Gal4 ; UAS-Mi2 RNAi
ns
a10
CG15446
vas
B
wor-GAL4> mCherry RNAi
wor-GAL4> Mi-2 RNAi
Dlg Dpn
C
Neuroepithelial cell score
n = 7
n = 8
Control
Mi-2 RNAi
wor-GAL4 (NSCs)
D
Larval crawling speed (cm/min)
ns
wor-GAL4> mcherry RNAi
wor-GAL4> Mi-2 RNAi
E
elav> mcherry RNAi (control)
elav> Mi-2 RNAi with repo-GAL80
Dpn Elav
Elav
Dpn
F
Dlg Dpn
Control
elav-GAL4> Mi-2 RNAi

Figure EV4.

Figure EV4. Differential requirements of Mi-2 during neuronal differentiation.

A Knockdown of *Mi-2* in adult brain neurons does not cause ectopic expression of genes. qPCR measurement of gene expression in adult heads. *Mi-2*^{RNAi} is induced for 24 h using GAL80^{ts}. No significant changes in expression of either *vas*, *a10* or *CG15446* were observed (one-tailed students *t*-test, represented as mean ± SEM.). Three biological replicates per genotype.

B Representative images of optic lobes for control and *Mi-2* knockdown in NSCs.

C Quantification of optic lobe neuroepithelial cells of genotypes shown in panel B ($n >= 7$ for each group). Represented as a box plot. No significant difference found (one-tailed student's *t*-test).

D Larval crawl speed (cm/min) in *wor*-GAL4 × *Mi-2* RNAi and *wor*-GAL4 × *mCherry* RNAi controls. No significant changes in crawl speed were observed (two-tailed students *t*-test, $n = 10$ animals per genotype). Represented as a box plot.

E Optic lobe phenotype is not due to *Mi-2* knockdown in glial cells. Optic lobe of third instar larvae in which *repo*-GAL80 represses glial GAL4 expression. Loss of neuroepithelial cells is still observed as in *elav*-GAL4; *Mi-2 RNAi* brains. Scale bar = 50 μm.

F Characteristic rosette structure of the neuroepithelial cells is present in the control first instar optic lobe and in *elav*-GAL4; *Mi-2 RNAi* 1st instar optic lobes. Scale bar = 20 μm.

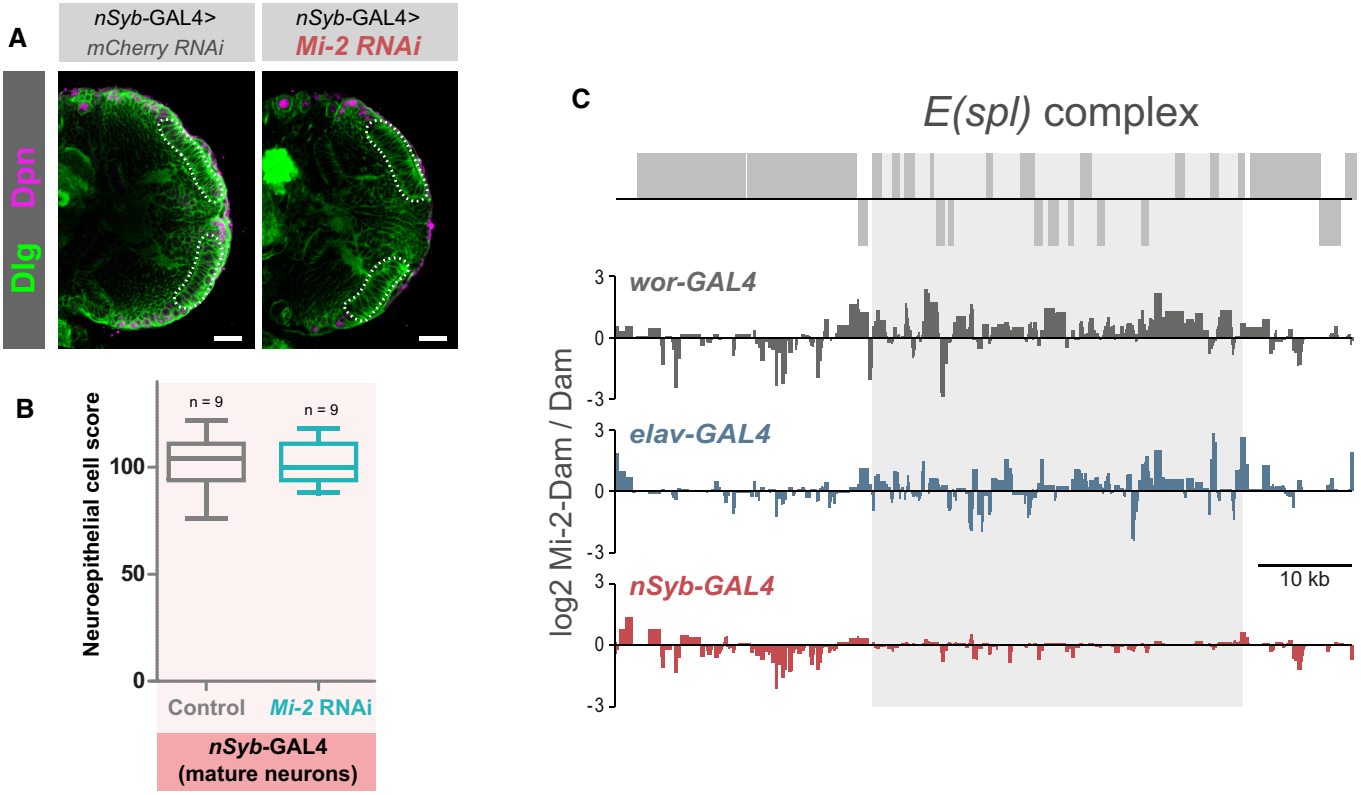

Figure EV5. Differential requirements and binding of Mi-2 during neuronal differentiation.

A Representative images of optic lobes for control and *Mi-2* knockdown in mature neurons.

B Quantification of optic lobe neuroepithelial cells of genotypes shown in panel B ($n >= 7$ for each group). Represented as a box plot. No significant difference found (one-tailed student's *t*-test).

C Mi-2 binding at the *E(spl)* complex is lost in mature neurons. Grey boxes represent genes.